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Herbivory by the insect *Diaphorina citri* induces greater change in citrus plant volatile profile than does infection by the bacterium, *Candidatus* Liberibacter asiaticus

Faraj Hijaz, Ibrahim El-Shesheny, and Nabil Killiny*

Department of Entomology and Nematology; Citrus Research and Education Center; University of Florida; IFAS; Lake Alfred, FL USA

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The volatile organic compound (VOC) profile in plant leaves often changes after biotic and abiotic stresses. Monitoring changes in VOCs in plant leaves could provide valuable information about multitrophic interactions. In the current study, we investigated the effect of Asian citrus psyllid (ACP) infestation, citrus greening pathogen (*Candidatus* Liberibacter asiaticus [*C*Las]) infection, and simultaneous attack by ACP and *C*Las on the VOC content of citrus leaves. Leaf volatiles were extracted using hexane and analyzed with gas chromatography-mass spectrometry (GC-MS). Although ACP is a phloem-sucking insect that causes minimal damage to plant tissues, the relative amount of 21 out of the 27 VOCs increased 2- to 10-fold in ACP-infested plants. The relative amount of *d*-limonene, *β*-phelandrene, citronellal, and undecanal were increased 4- to 20- fold in *C*Las-infected plants. A principle component analysis (PCA) and cluster analysis (CA) showed that VOC patterns of ACP-infested and *C*Las-infected plants were different from each other and were also different from the controls, while the VOC pattern of double-attacked plants was more like that of the controls than that of ACP-infested or *C*Las-infected plants. VOC amounts from leaves were compromised when plants were attacked by ACP and *C*Las. The results of this study showed that a simple direct extraction of citrus leaf volatiles could be successfully used to discriminate between healthy and *C*Las-infected plants. Information about the effects of insect and pathogen attack on the VOC content profile of plants might contribute to a better understanding of biotic stress.

Introduction

The main functions of plant volatiles are to protect plants from insect and pathogen attack, attract pollinators and other beneficial animals, and serve as communication signals within plant and among plants.[1] Both biotic and abiotic stress can trigger plant volatiles.[2]

Plant volatiles are synthesized and stored in different sites such as glandular trichomes, secreting ducts and cavities, secretory cells in flowers and root, and in extra-floral nectar.[3] In *Citrus sp* L. species, essential oils are present in special oil glands in leaves, flowers, peel, juice vesicles, and seeds.[4] The composition of citrus leaf oils is mainly stable and shows small variation from one season to another.[5] In addition, it is slightly affected by the rootstock.[4]

The Asian citrus psyllid (ACP), *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae), is the main vector of the citrus greening pathogen (*Candidatus* Liberibacter asiaticus [*C*Las]), a phloem-limited, uncultivable, and gram-negative bacterium.[6] Recent studies using 16S rRNA gene sequencing showed that 3 species of the bacterium, *Candidatus* Liberibacter, have been implicated in citrus greening: asiaticus (*C*Las)(in Asia, North America, and Brazil), africanus (*C*Laf) (in Africa), and americanus (*C*Lam) (in Brazil).[7] *C*Las and *C*lam are transmitted by ACP, while *C*Laf is transmitted by the African citrus psyllid *Trioza erytrea* (Del Guercio) (Hemiptera: Triozidae).[8] The ACP transmits the HLB pathogen during feeding activities on citrus phloem sap.

Most studies on the insect-plant interactions focused on volatiles released from herbivore-damaged plants.[9] Rupturing of the storage glands by insect feeding causes immediate release of stored volatiles.

Volatiles released from herbivore-damaged plants are distinctively different from those released from mechanically damaged plants.[10] Mechanical damage of cotton leaf induces glands to release stored terpenes and induced emissions of green-leaf volatiles (GLVs). Some volatiles like indole and hexenyl acetate are released in higher levels in herbivore-damaged plants than

Correspondence to: Nabil Killiny; Email: nabilkilliny@ufl.edu

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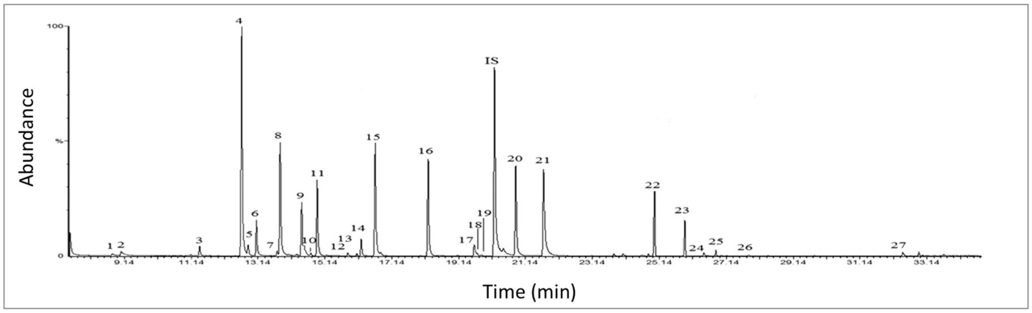

Figure 1. GC-MS chromatogram of healthy Valencia leaf hexane extract. The numbers above the peaks refer to the numbers listed in **Table 1**. IS: internal standard used to calculate the absolute amount of each compounds.

mechanically damaged plants.[11] Volatile emissions and plant defense are triggered by enzymes and elicitors from insect herbivores.[10,12]

Volatiles from herbivore-attacked plant are released immediately or synthesized after several hours or even days after attack.[9] In general, plant responses differentially to chewing insects than piercing-sucking insects. In the case of chewing insects, damaged tissue release GLVs that are produced immediately after insect damage.[13] GLVs contain C6 aldehydes, alcohols, and esters such as hexanal, hexenol, and hexyl acetate, respectively. Piercing-sucking insects cause release of stored volatiles after enhancing by elicitors.

Volatiles released from herbivore-damaged plants may result in direct or indirect defense against herbivores; deterring herbivores as well as attracting natural enemies such as arthropod predators, parasitoids, and birds.[14] Plant volatiles released by herbivore-damaged plant can also transmit information within plant and potentially among plants.[1] For example, laboratory studies showed that the expression of several genes involved in defense metabolism were elevated in *Phaseolus vulgaris* (lima bean) that was exposed to volatiles from herbivore-infested neighbors.[15]

Released volatiles are usually collected using a closed push/pull system. In this method, volatiles are pulled out through a trap that contains specific adsorbent. The collected volatiles are eluted with an organic solvent and analyzed using GC-MS.[16-18] Although this procedure is simple and does not require sample cleanup, the type and the size of the adsorbent, collecting time, and collecting rate affect the quantity and quality of collected volatiles. Accordingly, the results obtained by this method may not reflect the actual released volatiles.

Studying the emission of plant volatiles in response to biotic stress is important because they are involved in direct and indirect defense. However, studying the total volatile contents of plant leaves after the attack might contribute to a better understanding of plant response to pathogen and insect attack, mechanism of volatile release, and the relationship between stored and released volatiles.

Because volatile extraction from plant leaves is difficult and require purification, in some studies volatile contents of plant leaves were collected from grounded samples using purge and trap apparatus[19] or were extracted with solid phase micro-extraction (SPME).[20]

Available information about the response of citrus leaf volatiles to ACP feeding and *C*Las infection is limited and only 2 reports have considered the response of citrus leaf volatiles to *C*Las infection. In first report, researchers used solid-phase microextraction (SPME) coupled with (GC-MS) to study the effect of *C*Las on citrus leaf volatiles.[20] In second study, they used GC-MS to identify citrus leaf volatiles that were released in the headspace of *C*Las-infected citrus trees and studied their effect on psyllid behavior.[18] The objective of this study was to determine the effect of ACP infestation, *C*Las infection, and the double attack of ACP and *C*Las on the volatile content of citrus leaves.

Materials and Methods

Insect colonies and citrus tree maintenance

Field-collected ACP adults were used to establish insect colonies. Colonies were maintained in controlled growth rooms at 28 ± 1 °C, 60 ± 2% RH, and under an L16:D8 h photoperiod. Eight-month-old seedlings of Valencia sweet orange (*Citrus sinensis* (L.) Osbeck) were used in this study when we initiated the experiment. Trees were maintained in an insect-proof, AC-controlled greenhouse (28 °C, 40% RH, L16:D8). To obtain infected trees with *C*Las, 8-mo-old sweet orange seedlings from Valencia were grafted with 4 pieces of budwood sticks from a PCR-positive HLB source. Eight months later, the infection was confirmed using PCR as described by Tatineni et al.[21] PCR was also used to check for the presence of *C*Las in citrus trees and to insure that ACP colonies were negative from *C*Las.

Tree exposure to Asian citrus psyllid (*Diaphorina citri*)

Eighteen-month-old healthy or *C*Las-infected Valencia seedlings were experimentally exposed to healthy psyllids. Each tree was challenged with 50 adults and individually caged using insect rearing cages (30 × 15.5 × 15 inches). Exposed trees were kept in growth rooms under the condition as described before. Five months post exposure; 6 mature leaves were collected from each tree from different positions for further analysis. Control (healthy) and *C*Las-infected trees without any exposure to ACP were also kept under the same condition in separate cages. For each of the 4 treatments, 6 replications have been used.

Extraction of citrus leaf volatiles

Leaves were ground to a fine powder with liquid nitrogen and 100 mg was transferred to a 1.5 ml micro-centrifuge tube.

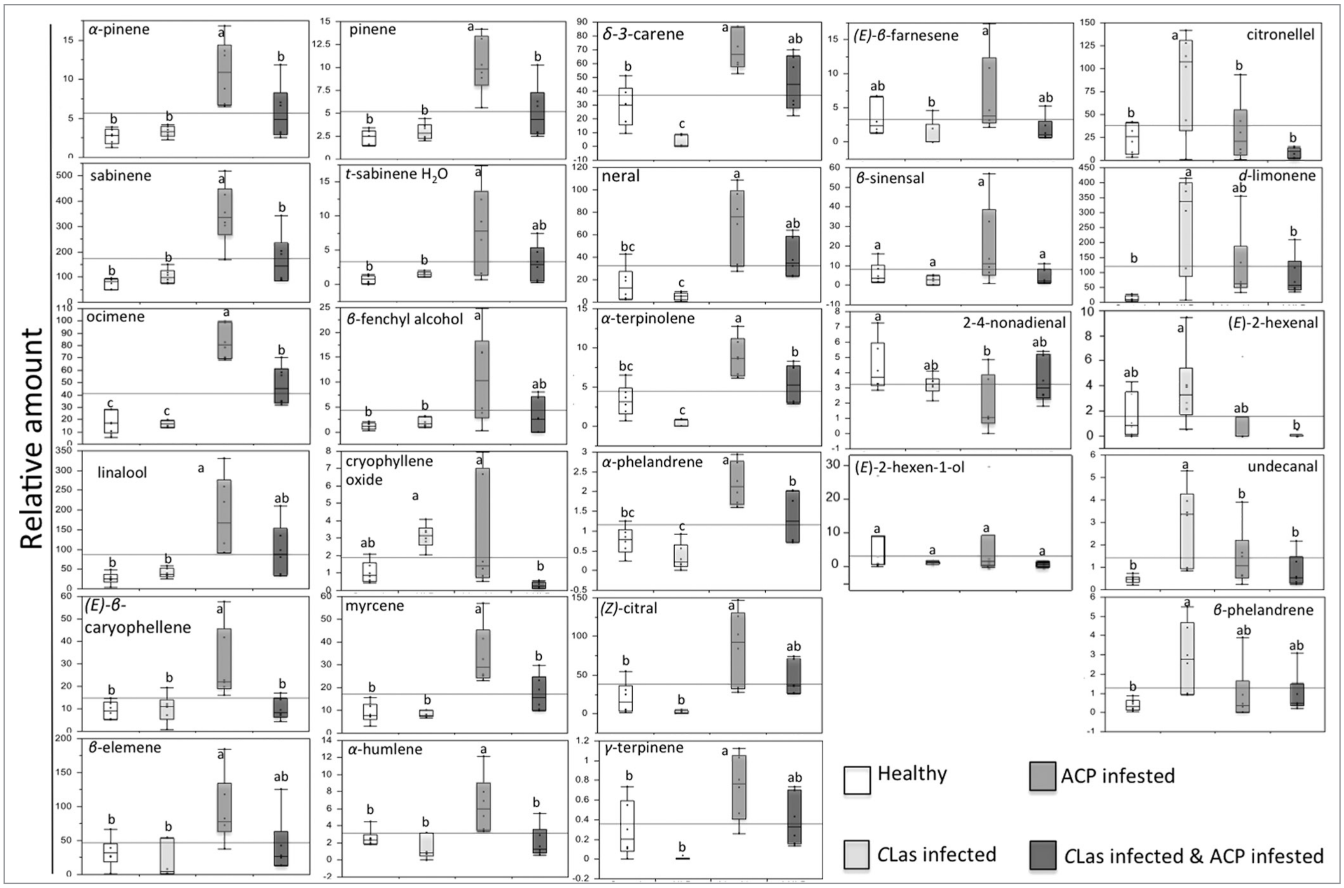

Figure 2. Relative amount of volatile compounds in different treatment. The peak areas were normalized using internal standard (*trans, trans*-2,4-nonadienal). Horizontal thick lines indicate the medians; boxes show the interquartile ranges including 50% of the values; whiskers reflect the highest and the lowest number of visits; and different letters indicate statistically significantly different treatments ($P < 0.05$).

A 0.5-ml aliquot of hexane (Sigma) was added to the samples and vortexed for 30 sec. Samples were left on ice for 10 min and the vortexing was repeated 2 times. At the end of the extraction, samples were removed from the ice and centrifuged at 12,000 rpm for 1 min. A 0.2-ml aliquot of the organic layer was spiked with *trans*, *trans*-2,4-nonadienal (Sigma) as internal standard at a final concentration of 200 ppm, and 1 µl of the spiked sample was injected into the GC-MS running in the full scan mode. Overall, 6 plants and 6 leaves from each were used for each treatment and two runs/replicate were performed.

GC-MS analysis

Citrus leaf volatiles were analyzed using a Clarus 500 GC-MS system (Perkin Elmer) fitted with an HP-5MS column (cross-linked 5% Ph Me siloxane, 50 min × 0.22 mm × 0.025 µm film thickness). The flow rate for the helium carrier gas was 0.7 ml/min. The following GC temperature program was used: hold at 50 °C for 3 min, then increase to 200 °C at a rate of 5 °C/min, increase further to 250 °C at 10 °C/min, and finally hold at 250 °C for 2 min.[22] The injector and the detector temperatures were 250 °C and 180 °C, respectively.

Peak identification and quantification

GC-MS chromatograms were analyzed using TurboMass software version 5.4.2 (Perkin Elmer). Peak identifications were achieved using NIST (Natl. Inst. of Standards and Technology) and Wiley 9th edition (John Wiley and Sons, Inc.,) mass spectra database libraries and linear retention index (LRI). The LRI values were calculated using a calibration curve generated by injecting a mixture of alkane (C8–C18) under previously described conditions. The percentage peak areas were calculated by dividing the peak area of each compound by the total area. To fairly compare the relative amount of each compound in different treatments, we normalized the peak areas using *trans*, *trans*-2,4-nonadienal as the internal standard (IS). The compounds' amounts were normalized by dividing their peak areas by IS peak area.

Statistical analyses

Statistical analyses were performed using JMP version 9.0 (SAS Institute Inc,). Data were normally distributed. Analysis of variance (ANOVA) was used to compare estimated volatiles in different treatments as a percentage (proportion) or normalized (amounts). Post hoc pairwise comparisons between treatments were performed with the Tukey honestly significant different test. Principal component analysis (PCA) and cluster analysis (CA) were used to discriminate among treatments. The PCA and CA were performed using the normalized data of individual VOCs and 3 main groups. PCA was used to summarize the pattern of correlation among the detected compounds in the

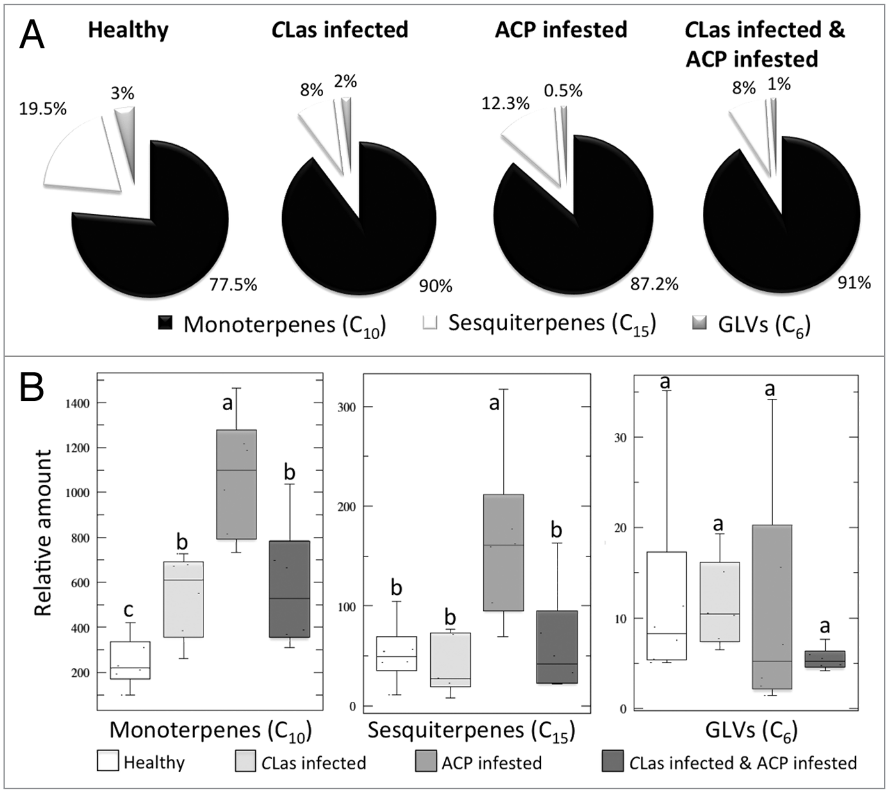

Figure 3. Proportions (percentages) and relative amount of volatile compounds categorized into groups in hexane extract of Valencia leaves. (**A**) Proportions (percentage). Number followed by each compound represents its percentage. (**B**) Relative amounts. Horizontal lines indicate the medians; boxes show the interquartile ranges including 50% of the values; whiskers reflect the highest and the lowest number of visits; and different letters indicate statistically significantly different treatments ($P < 0.05$).

different treatments. CA based on the Mahalanobi's squared distance between groups from the discriminant function analysis (DFA), with 95% confidence, was used to construct the similarity dendograms.

Results

Volatile profile in Valencia orange leaf

In order to study the effect of ACP-infestation and/or *C*Las infection on the composition of volatile components in citrus leaves, we used hexane to extract the total volatiles. The composition of Valencia leaf individual volatiles is presented in **Table 1** and the corresponding chromatogram is shown in (**Fig. 1**). Overall, 27 compounds were detected and identified in Valencia leaf. The main components were sabinene 26.2%, β-elemene 11%, δ-3-carene 9.1%, linalool 8.4%, citronellal 7.4%, ocimene 5.7%, (*Z*)-citral 6.3%, and neral 4.4% (**Table 1**).

Citrus responses systemically to biotic stress

In order to examine whether citrus plants respond systemically to the infection with *C*Las, the infestation with ACP or both together, leaves from different locations in plants were analyzed. No changes in volatile profile or volatile relative amounts were found among all tested leaves within each treatment. Additionally, for *C*Las-infected plants, no differences were found between symptomatic and asymptomatic leaves (data non shown). Data were pooled in further analyses. This result suggests a systematic response within the plant to the biotic stresses.

Infection with *C*Las and/or infestation with ACP alter the volatile proportions profile

To compare the profile of different treatments, we calculated the proportion of each compound to all others for each treatment. The volatile profile of *C*Las-infected plants was more affected than that of ACP-infested or combined ACP and *C*Las. Four compounds were increased and 10 compounds were decreased in the profile of *C*Las-infected plants compared with control (healthy). The percentages of *d*-limonene, β-phellandrene, citronellal, and undecanal were significantly higher in leaves from *C*Las-infected plant. A dramatic increase in *d*-limonene percentage from 3.5 to 40.1% was observed. This observation could be used as a biomarker for fast identification of *C*Las-infected plants. A significant reduction in the percentage of (*E*)-2-hexen-1-ol, myrcene, α-phellandrene, δ-3-carene, γ-terpinene, α-terpinolene, 2,4-nonadienal, neral, (*Z*)-citral, and α-humulene was found in *C*Las-infected plants (**Table 1**).

The volatile profile of leaves from plants infested with ACP was relatively similar to the control profile with the exception of two compounds that were increased (linalool and *d*-limonene) and five compounds that were decreased [(*E*)-2-hexen-1-ol, (*E*)-2-hexenal, 2,4-nonadienal, citronellal, and δ-3-carene] (**Table 1**).

The volatile profile of attacked plants by ACP and *C*Las was more affected than ACP-infested plants and less affected than those infected by *C*Las. Seven compounds were decreased and two compounds were increased significantly within the profile. The percentages of the C6-GLVs [(*E*)-2-hexen-1-ol, (*E*)-2-hexenal, and 2,4-nonadienal], citronellal, (*E*)-β-caryophllene, α-humelen, and caryophyllene oxide were significantly lower, while the percentage of linalool and *d*-limonene was higher in the double-attacked plants (**Table 1**).

Infection with *C*Las and/or infestation with ACP alter volatile amounts

The intensive increase in *d*-limonene percentage (3.5 to 40%) in the *C*Las-infected plants affected the proportion of compounds to each other. Subsequently, the profile of *C*Las-infected looked significantly different from those of the healthy or the ACP-infested plants. To compare the relative amount of each compound in different treatments, we used the normalized data as described above. Unlike the profiles, we found that most compounds responded to the infestation with ACP, while only a few of them responded to the infection with *C*Las. The compounds

Table 1. Proportions (percentages) of of VOCs detected in healthy, *C*las-infected, ACP-infected, and double-attacked Valencia leaves

Group(1)	Compound	RI	Volatile proportion to each other (%)(2)							
			Healthy		*C*las-infected		ACP-infested		Double-attacked	
C_6-GLVs										
Alc.	(*E*)-2-hexen-1-ol	825	0.9	0.9 ± 0.2^{a}	0.3	0.3 ± 0.1^{b}	0.2	0.2 ± 0.0^{b}	0.2	0.2 ± 0.1^{b}
Ald.	(*E*)-2-hexenal	834	2.1	0.4 ± 0.2^{a}	1.6	0.5 ± 0.2^{a}	0.3	0.0 ± 0.0^{b}	0.8	0.0 ± 0.0^{b}
	2,4-nonadienal	1222		1.5 ± 0.6^{a}		0.6 ± 0.2^{b}		0.2 ± 0.2^{b}		0.7 ± 0.5^{b}
	undecanal	1230		0.2 ± 0.1^{b}		0.5 ± 0.2^{a}		0.1 ± 0.1^{b}		0.1 ± 0.1^{b}
Total C_6-GLVs			3.0		1.9		0.5		1.0	
Monoterpenes (C_{10})										
Mt. alc.	β-fenchyl alcohol	1224	8.8	0.4 ± 0.3^{a}	7.6	0.3 ± 0.2^{a}	15.4	0.8 ± 0.7^{a}	15.0	0.4 ± 0.4^{a}
	Linalool	1113		8.4 ± 1.8^{b}		7.3 ± 3.6^{b}		14.6 ± 5.4^{a}		14.6 ± 4.9^{a}
Mt. ald.	Citronellal	1171	18.1	7.4 ± 1.8^{b}	15.1	14.0 ± 4.0^{a}	15.2	2.5 ± 2.2^{c}	15.8	1.7 ± 1.5^{c}
	Neral	1267		4.4 ± 3.2^{a}		0.8 ± 0.4^{b}		5.7 ± 2.5^{a}		6.6 ± 2.1^{a}
	(*Z*)-citral	1297		6.3 ± 4.4^{a}		0.3 ± 0.4^{b}		7.0 ± 3.6^{a}		7.5 ± 2.6^{a}
Mt. est.	*t*-sabinene H2O	1082	0.2	0.2 ± 0.2^{b}	0.3	0.3 ± 0.1^{b}	0.6	0.6 ± 0.2^{a}	0.5	0.5 ± 0.1^{a}
Mt. hd.	α-pinene	919	50.4	0.9 ± 0.2^{a}	66.9	0.6 ± 0.3^{a}	56.0	0.9 ± 0.2^{a}	59.6	0.8 ± 0.1^{a}
	Sabinene	965		26.2 ± 10.2^{a}		19.8 ± 13.1^{a}		27.8 ± 2.9^{a}		25.0 ± 2.7^{a}
	Pinene	973		0.8 ± 0.3^{a}		0.6 ± 0.4^{a}		0.8 ± 0.1^{a}		0.8 ± 0.1^{a}
	Myrcene	982		2.8 ± 0.6^{a}		1.5 ± 0.4^{b}		2.7 ± 0.8^{a}		2.7 ± 0.3^{a}
	α-phellandrene	1005		0.2 ± 0.1^{a}		0.1 ± 0.0 b		0.2 ± 0.0^{a}		0.2 ± 0.1^{a}
	δ-3-carene	1008		9.1 ± 1.9^{a}		0.7 ± 1.1^{c}		6.0 ± 2.3^{b}		7.4 ± 1.7^{ab}
	d-limonene	1032		3.5 ± 1.7^{b}		40.1 ± 22.1^{a}		9.7 ± 8.9^{b}		13.8 ± 7.8^{b}
	β-phellandrene	1042		0.1 ± 0.1^{b}		0.4 ± 0.2^{a}		0.1 ± 0.1^{b}		0.1 ± 0.1^{b}
	Ocimene	1049		5.7 ± 2.1^{ab}		3.0 ± 1.3^{b}		7.0 ± 2.1^{a}		7.9 ± 1.7^{a}
	γ-terpinene	1093		0.1 ± 0.1^{a}		0.0 ± 0.0^{b}		0.1 ± 0.0^{ab}		0.1 ± 0.0^{ab}
	α-terpinolene	1098		1.0 ± 0.3^{a}		0.1 ± 0.1^{b}		0.7 ± 0.2^{a}		0.8 ± 0.2^{a}
Total monoterpenes			77.5		90		87.2		91	
Sesquiterpenes (C_{15})										
Sqt. hd.	β-elemene	1419	19.1	11.6 ± 9.0^{a}	7.7	4.8 ± 6.9^{a}	12.1	7.3 ± 2.4^{a}	8.1	5.5 ± 2.8^{a}
	(*E*)-β-caryophyllene	1452		3.0 ± 0.6^{a}		1.8 ± 0.9^{ab}		2.4 ± 1.0^{ab}		1.6 ± 0.3^{b}
	(*E*)-β-farnesene	1473		1.4 ± 1.5^{a}		0.3 ± 0.5^{a}		0.5 ± 0.3^{a}		0.2 ± 0.1^{a}
	α-humulene	1486		0.9 ± 0.4^{a}		0.3 ± 0.4^{b}		0.5 ± 0.1^{ab}		0.3 ± 0.1^{b}
	β-sinensal	1691		2.2 ± 2.1^{a}		0.5 ± 0.5^{a}		1.4 ± 1.2^{a}		0.5 ± 0.4^{a}
Sqt. ox.	caryophyllene oxide	1521	0.4	0.4 ± 0.3^{ab}	0.5	0.5 ± 0.2^{a}	0.2	0.2 ± 0.2^{bc}	0	0.0 ± 0.0^{c}
Total sesquiterpenes			19.5		8		12.3		8.1	

(1)Abbreviations of groups: Alc, Alcohol; Ald, Aldehyde; Mt. alc, Monoterpene alcohol; Mt. ald., Monoterpene aldehyde; Mt. est., Monoterpene esters; Mt. hd., Monoterpene hydrocarbon; Sqt. hd., Sesquiterpene hydrocarbon; Sqt. ox., Sesquiterpene oxide. (2) Numbers that share the same superscript letters, in the same row, are not statistically different at 95% level of confidence.

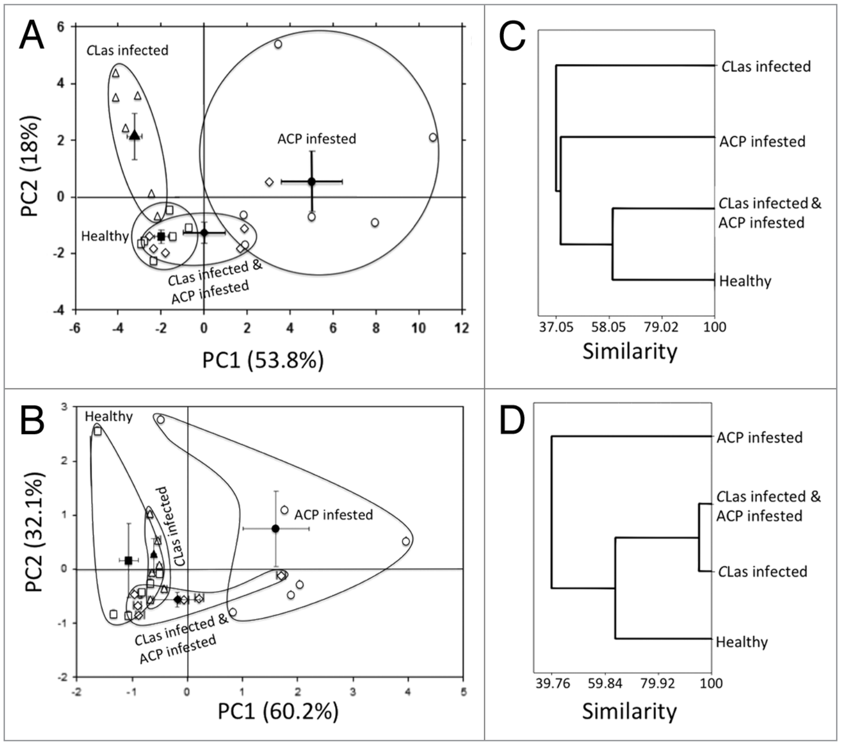

Figure 4. Principal components and cluster analysis of volatile organic compounds in different treatments (**A**) Principal component analysis of individual components. (**B**) Principle component analysis of volatile compounds categorized into main groups (monoterpenes, sesquiterpenes, and green leaf volatiles). (**C**): Cluster analysis using the individual compounds. (**D**) Cluster analysis of volatile compounds categorized into main groups (monoterpenes, sesquiterpenes, and green leaf volatiles).

were divided into two groups depending on their response to ACP or *C*Las as described below.

Specific response to ACP

The first group included 21 volatile compounds that were affected in ACP-infested plants (**Fig. 2**). Seventeen of these compounds were significantly higher. Fourteen out of the 17 were monoterpenes (*α*-pinene, sabinene, pinene, myrcene, ocimene, *t*-sabinene H2O, *γ*-terpinene, linalool, (*Z*)-citral, δ-3-carene, *α*-phelandrene, *α*-terpinolene, neral, and *β*-fenchyl alcohol) and the other 3 were sesquiterpens (*β*-elemene, *α*-humlene, and (*E*)-*β*-caryophllene). The abundance of these compounds increased between 2 to 10-fold (**Fig. 2**). None of these compounds were increased in *C*Las-infected plants. The amounts of these volatiles in *C*Las-infected trees that were also attacked by ACP were lower than those exposed to ACP only, and higher than those infected with *C*Las. This finding suggests a compromising effect due to the double attack (**Fig. 2**). The other 4 compounds displayed non-significant trends were caryophyllene oxide, *β*-sinensal, and (*E*)-*β*-farnesene (increased), and 2,4, nonadienal (decreased).

Specific response to *C*Las

Four volatiles were induced at significantly higher amounts in *C*Las-infected plants compared with the control (**Fig. 2**). These compounds were three monoterpenes (*d*-limonene, *β*-phelandrene, and citronellal) and an aldehyde (undecanal). These compounds were higher in *C*Las-infected plants than ACP-infested plants. The amounts of *β*-phelandrene, citronellal, and undecenal were induced 4-fold, while *d*-limonene induced to more than 20-fold (**Fig. 2**). Surprisingly, the amount of *δ-3*-carene, known for its antimicrobial activity, was decreased significantly in *C*Las-infected plants. We also observed that the double attack compromised the amount of these compounds. As expected, the C_6-GLVs [*(E)*-2-hexenal and *(E)*-2-hexen-1-ol] were not significantly affected.

The effect of *C*Las and/or infestation with ACP on volatiles as categories

The 27 detected compounds were classified into 3 main categories; C6: GLVs, C10: monoterpenes, and C15: sesquiterpenes (**Table 1** and **Fig. 3**). The percentages of these categories in healthy leaves were 3%, 77.5%, and 19.5%, respectively. These 3 categories consisted of 8 groups based on their functional group. C6-GLVs included 2 groups: Alcoholic (0.9%) and aldehyde (2.1%) compounds. Monoterpenes include monoterpenes hydrocarbon (50.4%), monoterpenes aldehyde (18.1%), monoterpene alcohol (8.8%), and monoterpene esters (0.2%). The 2 functional groups of sesquiterpenes were sesquiterpene hydrocarbons (19.1%) and sesquiterpene oxide (0.4%).

The monoterpenes category was increased significantly in all treatments while both C6-GLVs and sesquiterpenes were decreased (**Fig. 3A**). The percentage of monoterpene hydrocarbon increased to 66.9, 56.0, and 59.6% in the *C*Las-infected, ACP-infested, and double-attacked leaves, respectively. These increases were due to the great increase in *d*-limonene and *β*-phellandrene in *C*Las-infected and *d*-limonene, sabinene, and ocimene in ACP-infested and double-attacked plants. Finally, the percentages of monoterpene alcohols, especially linalool, were increased in ACP-infested and double-attacked plants but remained constant in *C*Las-infected plants compared with the control.

Changes of the relative amounts of the main group due to *C*Las-infection and/or ACP-infestation are shown in **Figure 3B**. There was a significant increase in monoterpenes in all treatments compared with control. ACP-infested plants possessed a higher amount of monoterpenes than *C*Las-infected and double attacked plants. No significant differences in the amount of monoterpenes were observed between *C*Las infected and double attacked. Sesquiterpenes increased significantly only in ACP-infested plants. No significant differences among treatments were found in C6-GLVs.

Clustering and multivariate analysis

PCA using the individual VOCs showed clear clustering among treatments and we were able to discriminate between *C*Las-infected and ACP-infested plants (**Fig. 4A**), The principal components (PC) 1 and 2 accounted for 71.1% of the variation. Pinene, *α*-pinene, sabinene, myrcene, *α*-phellandrene, ocimene, *t*-sabinene H2O, α-terpinolene, linalool, neral, and (*Z*)-citral were the compounds with the highest absolute loading values in PC1. While *d*-limonene, β-phellandrene, citronellal, undecanal, (*E*)-β-caryophyllene oxide, and (*E*)-β-caryophyllene were among the compounds with the highest absolute PC 2 loading. In addition, PCA using the three main groups of VOCs showed a clear discrimination between control and ACP-infested plants (**Fig. 4B**). The principal components (PC) 1 and 2 accounted 92.2% of the variation. Monoterpenes has the highest sum of the absolute PC 1 and 2 loading, followed by sesquiterpenes. Because VOC patterns of the samples in each treatment were clustered around their centroid, this supports our findings that plant responses to ACP and *C*Las attack were systematic.

Likewise PCA and CA using individual VOCs or main groups showed that VOCs profile in the leaves from double-attacked plants was closer to the profile of *C*Las-attacked plants than ACP-attacked plants (**Fig. 4C and D**). CA using the 3 main categories showed high similarity between *C*Las-infected and double-attacked plants (95.13%) (**Fig. 4D**). The ACP-infested plants were more distinguished from the control (39.76% similarity) than *C*Las-infected and double-attacked plants (63.45%) (**Fig. 4D**). CA analysis using individual volatiles showed that VOCs profile in the leaves from double-attacked plants was closer to the profile of *C*Las-attacked plants than ACP-attacked plants (**Fig. 4C**). VOCs in double-attacked plants were closer to the control (59.61% similarity) than the ACP-infested (39.01% similarity) or *C*Las-infected (37.05% similarity) (**Fig. 4C**).

Discussion

Twenty-seven VOCs were successfully identified and quantified. Most of the compounds detected in our study were reported to be released by citrus leaf.[18,23] No significant changes in volatile profile or volatile relative amounts were found among leaves from different positions within the plant in each treatment suggesting a systemic response in citrus plants as previously described.[24]

In our study, the major group of VOCs in leaf from healthy plants was terpenes (97%), while C6-GLVs represented only 3%. Terpenoids represent the largest VOCs in most plant leaves.[9,25] Both monoterpenes and sesquiterpenes protect plants against biotic or abiotic stresses.[24,26]

Plant infestation by herbivores induces compounds implicated in antixenosis or antibiosis,[27] and attracts natural enemies.[28] In addition, the infection with pathogens induces the production of antimicrobial compounds.[29] In general, terpenoids play an important role in plant-herbivore-pathogen interaction.[30] Many plants show high emission of monoterpenes after herbivory and/or pathogen attack. Monoterpenes may act as kairomones in attracting predators and parasitoids to attacked plants,[14] as well as function as phytoalexins,[31] affect oviposition behavior,[32,33] and act as feeding deterrents for insects.[34] Monoterpenes and sesquiterterpenes were found to be induced systemically in Pinus sylvestris by egg deposition of the sawfly *Diprion pini*.[24]

As expected, in our results, there were no significant differences in the abundances of GLVs (C6) between ACP-infested and/or CLas-infected and control plants. In fact, the plant bleeds GLVs instantaneously from disrupted tissue as a wounding response, but AC P causes minimal damage to plant tissue; therefore, no effect on GLVs was found.

Plant response for piercing-sucking insects such as aphids, mealy bugs, leafhoppers, and psyllids is different than that for chewing insects.[35] Although piecing-sucking insects do not cause significant cell damage, during their prolonged stylet interactions, injected elicitors might induce specific reactions.[36] In general, chewing insects induce the expression of jasmonic acid pathway (JA) regulated gene and wound-responsive gene while the piercing-sucking insects such as ACP induces salicylic acid (SA) signaling pathway[3] and JA pathway.[36] In ACP-infested leaves, 17 compounds were significantly higher in ACP-infested plants. Attack with other species of psyllids induces many changes in leaf VOCs. For example, Psylla pyricola feeding causes simultaneous changes in the composition of released and stored VOCs in pear trees.[37] Relative abundance of secondary metabolite volatiles in the leaves of avocado was correlated positively with psyllid (*Trioza anceps*) attack.[38] Aphids also induce the release of VOCs including α-pinene, β-pinene, cymene, α-phellandrene, and *d*-limonene.[39]

In our study, monoterpene alcohol in ACP-infested and double-attacked plant leaves was changed from 8.8% to 15.4%. The main volatile compound of monoterpene alcohol was linalool, which increased 7-fold compared with the control. In fact, linalool is a cue molecule that helps insects locate and recognize plants.[40] Linalool synthase in tomato was induced in leaf trichomes by spider mite infestation.[41] Linalool and ocimene were induced in lima bean after spider mite attack.[42] Ocimene was increased in ACP-infested and double-attacked but not in *C*Las-infected leaves as compared with the control. It seems that to induce ocimene, plants need several events such as several feeding periods by insect. A study supporting this idea showed that a single event of damage for Lotus japonicus plants did not induce the release of (*E*)-β-ocimene from the plants.[43] The monoterpene myrcene, was also increased in ACP-infested plants. This observation was found in Arabidopsis infested by P. rapae.[44] α-humulene, (*E*)-β-caryophyllene, and caryophyllene oxide were increased as a response to ACP-infestation. Both α-humulene and caryophyllene oxide were also induced in response to simultaneous infestation with sap-feeder insect, Frankliniella occidentalis, and chewing insect, Heliothis virescens.[45]

Some induced volatiles in ACP-infested plants were reported to be biologically active and attractive for natural enemies such as sabinene and α-pinene.[46] (*E*)-β-caryophyllene was increased in ACP-infested plants. The release of (*E*)-β-caryophyllene attracts entomopathogenic nematodes to Diabrotica virgifera larvae around maize roots.[47]

Plants infested with sap-feeder insects such as aphids are induced to release VOCs that attract parasitoid wasps[48] and determine aphid densities on host plants.[49] β-ocimene, linalool, α-farnesene, β-farnesene were induced by corn leaf aphid feeding.[10,49] Our findings revealed that (*E*)-β-farnesene was higher in ACP-infested than *C*Las-infected plants and control. (*E*)-β-farnesene is an aphid alarm pheromone and is also released from aphid-infested plants to call parasitoids.[48]

α-pinene was found to repel spruce beetle from *Pinus resinosa* plants and enhance predator's attraction.[50] In our study, *α*-pinene increased 5-fold in ACP-infested plants compared with control. The monoterpenes *p*-cymene, *α*-terpinene, and *α*-phellandrene repelled *Bemisia tabaci* when these compounds were applied on tomato.[51] *β*-sinensal was increased (4-fold) in ACP-infested plants. *β*-sinensal represents 9.4% of compounds in sandalwood oil. This oil produced mortality and deterrent effects against two-spotted spider mite, *Tetranychus urticae* Koch.[52] *(E)-β*-caryophyllene which increased 3-fold in ACP-infested plants compared with control was reported as attractant to newly emerged females of phloem-sap psyllid, *Cacopsylla picta*, the vector of *Candidatus* Phytoplasma mali in apple.[53] Sabinene was the highest compound in healthy trees and increased in all treatments especially in ACP-infested trees (more than 4-fold) and in double-attacked trees (more than 2-fold). (*E*)-ocimene, *d*-limonene, myrcene, and sabinene were the main volatiles produced by the new shoots of citrus that attract ACP.[23]

The infection with plant pathogens induces the production of plant volatiles that possess a powerful antimicrobial activity to inhibit the movement of the pathogens within plant tissues.[3,54] Although, the percentage profile of VOCs in *C*Las-infected plants was highly affected, only 4 compounds were increased in leaves from *C*Las-infected plants. These compounds include 3 monoterpenes (*d*-limonene, *β*-phelandrene, and citronellal) and an aldehyde (undecanal).

The accumulated compounds could play an important role in plant-pathogen interactions. In vitro studies showed that tomato leaf volatiles, including *β*-phelandrene and other compounds inhibited Botrytis cinerea fungus.[55] The antimicrobial activity of *d*-limonene was also assessed against 8 organisms.[56] It has been found that *C*Las-infected citrus plants released less *d*-limonene than healthy plants.[18] In current study, we showed an accumulation of *d*-limonene in leaves from *C*Las-infected plants. It could be a result of the induction of its biosynthesis and/or inhibition of its release. Theoretically, the level of any volatile in plant leaf is controlled by the rate of its formation and the rate of its loss (release or catabolism).[57] Volatiles could be released from storage glands or biosynthesized de novo following insect damage.[58]

A previous study showed that *C*Las-infected citrus plants were initially more attractive to D. citri adults than non-infected plants. However, after probing, psyllids moved to non-infected plants and settled.[18] Volatile cues may be implicated in the initial movement of psyllids to *C*Las-infected plants; while the arrestment is determined by the nutritional status of the host, a phenomenon was reported in other vector-borne diseases.[17,59] The results of the previous studies together indicate that the infection makes plants more attractive to insect-vectors by suppressing the plants basal defense, releasing attractive volatiles, or by inhibiting the release of deterrent volatiles. This will help in continuously spreading the disease by the vector. From the evolutionary view, there is a two-edge benefit for both the releaser and the receiver. First, for the releaser, it is a kind of resistance and may be used to invite natural enemies of ACP; second, for the receiver (ACP), this will direct the adults of insects to infest new trees, subsequently, less competition and increased distribution.

The accumulation of volatiles in citrus leaves induced by ACP attack was compromised when citrus plants were attacked simultaneously by *C*Las and ACP. It has been shown in other systems that the double biotic stress may compromise the plant response.[45] Plants attacked by more than 1 attacker produce some compounds that may induce a pathway and reduce the activity of another pathway and cause synergistic or antagonistic effects on other pathways. Pathogen-inducible SA causes down-regulation of the JA/ethylene-regulated defense-response genes, as well as JA-regulated wound responses and secondary metabolite accumulation.[60,61]

CA analysis using individual volatiles or main groups showed that VOCs profile in the leaves from double-attacked plants was closer to the profile of *C*Las-attacked plants than ACP-attacked plants. PCA of normalized data using individual volatiles or main group showed that double-attacked plants were closer to control than other treatments. These results prove that *C*Las infection compromises the responses of plant to ACP for its benefit.

Our findings suggest that *C*Las-infected and double-attacked plants might be more susceptible to other insects and pathogens since some biologically important compounds were decreased. These compounds possess antimicrobial activity or attraction to natural enemies. (*E*)-*β*-farnesene, the natural enemies attractant, decreased 3-fold in *C*Las-infected. (*E*)-2-hexenal and (*E*)-2-nonenal have an antimicrobial effect and are related to plant hypersensitive response.[62] Although, (*E*)-2-hexenal increased in *C*Las-infected plants and possesses high antibacterial properties even in low concentration,[62] it decreased severely in *C*Las-infected after attacking with ACP. (*E*)-2-hexenal and δ-3-carene, which has been reported as an antibacterial compound,[63] were reduced significantly in the infected plants with *C*Las. This suggests that the double attack of *C*Las and ACP in citrus makes plants more susceptible to other pests and pathogens.

Studying the effect of *C*Las and ACP in citrus plant volatile contents may lead to producing transgenic plants that over- or down-express certain compounds to enhance citrus resistance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Baldwin IT. Plant volatiles. Curr Biol 2010; 20:R392-7; PMID:20462477; http://dx.doi.org/10.1016/j.cub.2010.02.052
2. Arimura G, Matsui K, Takabayashi J. Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. Plant Cell Physiol 2009; 50:911-23; PMID:19246460; http://dx.doi.org/10.1093/pcp/pcp030
3. Maffei ME. Sites of synthesis, biochemistry, and functional role of plant volatiles. J Bot 2010; 76:612-31
4. Scora RW, Kumamoto J, Clerx WA. Variations of Leaf Oil Composition of Citrus Budded on Different Rootstocks. Syst Bot 1981; 6:31-7; http://dx.doi.org/10.2307/2418633
5. Hopfinger JA, Kumamoto J, Scora RW. Diurnal variation in the essential oils of Valencia orange leaves. Am J Bot 1979; 66:111-5; http://dx.doi.org/10.2307/2442513
6. Jagoueix S, Bove JM, Garnier M. The phloem-limited bacterium of greening disease of citrus is a member of the alpha subdivision of the Proteobacteria. Int J Syst Bacteriol 1994; 44:379-86; PMID:7520729; http://dx.doi.org/10.1099/00207713-44-3-379
7. Sagaram US, DeAngelis KM, Trivedi P, Andersen GL, Lu SE, Wang N. Bacterial diversity analysis of Huanglongbing pathogen-infected citrus, using PhyloChip arrays and 16S rRNA gene clone library sequencing. Appl Environ Microbiol 2009; 75:1566-74; PMID:19151177; http://dx.doi.org/10.1128/AEM.02404-08
8. Halbert SE, Manjunath KL. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: A literature review and assessment of risk in Florida. Fla Entomol 2004; 87:330-53; http://dx.doi.org/10.1653/0015-4040(2004)087[0330:ACPSPA]2.0.CO;2
9. Pichersky E, Gershenzon J. The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Curr Opin Plant Biol 2002; 5:237-43; PMID:11960742; http://dx.doi.org/10.1016/S1369-5266(02)00251-0
10. Paré PW, Tumlinson JH. Plant volatiles as a defense against insect herbivores. Plant Physiol 1999; 121:325-32; PMID:10517823; http://dx.doi.org/10.1104/pp.121.2.325
11. Loughrin JH, Manukian A, Heath RR, Turlings TC, Tumlinson JH. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plant. Proc Natl Acad Sci USA 1994; 91:11836-40; PMID:11607499; http://dx.doi.org/10.1073/pnas.91.25.11836
12. Mattiacci L, Dicke M, Posthumus MA. Induction of parasitoid attracting synomone in brussels sprouts plants by feeding of Pieris brassicae larvae: Role of mechanical damage and herbivore elicitor. J Chem Ecol 1994; 20:2229-47; http://dx.doi.org/10.1007/BF02033199
13. Gatehouse JA. Plant resistance towards insect herbivores: a dynamic interaction. New Phytol 2002; 156:145-69; http://dx.doi.org/10.1046/j.1469-8137.2002.00519.x
14. Kessler A, Baldwin IT. Defensive function of herbivore-induced plant volatile emissions in nature. Science 2001; 291:2141-4; PMID:11251117; http://dx.doi.org/10.1126/science.291.5511.2141
15. Arimura G, Ozawa R, Shimoda T, Nishioka T, Boland W, Takabayashi J. Herbivory-induced volatiles elicit defence genes in lima bean leaves. Nature 2000; 406:512-5; PMID:10952311; http://dx.doi.org/10.1038/35020072
16. Runyon JB, Mescher MC, De Moraes CM. Parasitism by Cuscuta pentagona attenuates host plant defenses against insect herbivores. Plant Physiol 2008; 146:987-95; PMID:18165323; http://dx.doi.org/10.1104/pp.107.112219
17. Mauck KE, De Moraes CM, Mescher MC. Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. Proc Natl Acad Sci USA 2010; 107:3600-5; PMID:20133719; http://dx.doi.org/10.1073/pnas.0907191107
18. Mann RS, Ali JG, Hermann SL, Tiwari S, Pelz-Stelinski KS, Alborn HT, et al. Induced release of a plant-defense volatile 'deceptively' attracts insect vectors to plants infected with a bacterial pathogen. PLoS Pathog 2012; 8:2-13; http://dx.doi.org/10.1371/journal.ppat.1002610
19. He PQ. Tian Li, Chen KS, Hao L, Li GY. Induction of volatile organic compounds of Lycopersicon esculentum Mill. and its resistance to Botrytis cinerea Pers. by burdock oligosaccharide. J Integr Plant Biol 2006; 48:550-7; http://dx.doi.org/10.1111/j.1744-7909.2006.00268.x
20. Cevallos-Cevallos JM, García-Torres R, Etxeberria E, Reyes-De-Corcuera JI. GC-MS analysis of headspace and liquid extracts for metabolomic differentiation of citrus Huanglongbing and zinc deficiency in leaves of 'Valencia' sweet orange from commercial groves. Phytochem Anal 2011; 22:236-46; PMID:21046688; http://dx.doi.org/10.1002/pca.1271
21. Tatineni S, Sagaram US, Gowda S, Robertson CJ, Dawson WO, Iwanami T, et al. *In planta* distribution of '*Candidatus* Liberibacter asiaticus' as revealed by polymerase chain reaction (PCR) and real-time PCR. Phytopathology 2008; 98:592-9; PMID:18943228; http://dx.doi.org/10.1094/PHYTO-98-5-0592
22. Lin SY, Roan SF, Lee CL, Chen IZ. Volatile organic components of fresh leaves as indicators of indigenous and cultivated citrus species in Taiwan. Biosci Biotechnol Biochem 2010; 74:806-11; PMID:20378980; http://dx.doi.org/10.1271/bbb.90891
23. Patt JM, Sétamou M. Responses of the Asian citrus psyllid to volatiles emitted by the flushing shoots of its rutaceous host plants. Environ Entomol 2010; 39:618-24; PMID:20388295; http://dx.doi.org/10.1603/EN09216
24. Mumm R, Schrank K, Wegener R, Schulz S, Hilker M. Chemical analysis of volatiles emitted by Pinus svlvestris after induction by insect oviposition. J Chem Ecol 2003; 29:1235-52; PMID:12857033; http://dx.doi.org/10.1023/A:1023841909199
25. Dudareva N, Pichersky E. Metabolic engineering of plant volatiles. Curr Opin Biotechnol 2008; 19:181-9; PMID:18394878; http://dx.doi.org/10.1016/j.copbio.2008.02.011
26. Keeling CI, Bohlmann J. Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. New Phytol 2006; 170:657-75; PMID:16684230; http://dx.doi.org/10.1111/j.1469-8137.2006.01716.x
27. Chen MS. Inducible direct plant defense against insect herbivores: A review. Insect Sci 2008; 15:101-14; http://dx.doi.org/10.1111/j.1744-7917.2008.00190.x
28. De Moraes CM, Lewis WJ, Pare PW, Alborn HT, Tumlinson JH. Herbivore-infested plants selectively attract parasitoids. Nature 1998; 393:570-3; http://dx.doi.org/10.1038/31219
29. Prisic S, Xu M, Wilderman PR, Peters RJ. Rice contains two disparate ent-copalyl diphosphate synthases with distinct metabolic functions. Plant Physiol 2004; 136:4228-36; PMID:15542489; http://dx.doi.org/10.1104/pp.104.050567
30. Dudareva N, Pichersky E, Gershenzon J. Biochemistry of plant volatiles. Plant Physiol 2004; 135:1893-902; PMID:15326281; http://dx.doi.org/10.1104/pp.104.049981
31. Howell CR, Hanson LE, Stipanovic RD, Puckhaber LS. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. Phytopathology 2000; 90:248-52; PMID:18944616; http://dx.doi.org/10.1094/PHYTO.2000.90.3.248
32. Delphia CM, Mescher MC, De Moraes CM. Induction of plant volatiles by herbivores with different feeding habits and the effects of induced defenses on host-plant selection by thrips. J Chem Ecol 2007; 33:997-1012; PMID:17415625; http://dx.doi.org/10.1007/s10886-007-9273-6
33. Wang H, Guo WF, Zhang PJ, Wu ZY, Liu SS. Experience-induced habituation and preference towards non-host plant odors in ovipositing females of a moth. J Chem Ecol 2008; 34:330-8; PMID:18253797; http://dx.doi.org/10.1007/s10886-008-9433-3
34. Laothawornkitkul J, Paul ND, Vickers CE, Possell M, Taylor JE, Mullineaux PM, et al. Isoprene emissions influence herbivore feeding decisions. Plant Cell Environ 2008; 31:1410-5; PMID:18643955; http://dx.doi.org/10.1111/j.1365-3040.2008.01849.x
35. Gosset V, Harmel N, Göbel C, Francis F, Haubruge E, Wathelet JP, et al. Attacks by a piercing-sucking insect (Myzus persicae Sultzer) or a chewing insect (Leptinotarsa decemlineata Say) on potato plants (Solanum tuberosum L.) induce differential changes in volatile compound release and oxylipin synthesis. J Exp Bot 2009; 60:1231-40; PMID:19221142; http://dx.doi.org/10.1093/jxb/erp015
36. Walling LL. The myriad plant responses to herbivores. J Plant Growth Regul 2000; 19:195-216; PMID:11038228
37. Scutareanu P, Drukker B, Bruin J, Posthumus MA, Sabelis MW. Leaf volatiles and polyphenols in pear trees infested by Psylla pyricola. Evidence of simultaneously induced responses. Chemoecology 1996; 7:34-8; http://dx.doi.org/10.1007/BF01240635
38. Rincon-Hernandez CA, Espinosa-Garcia FJ. Relationship between the phytochemical diversity in creole avocado (Persea americana var. drymifolia) leaves and the incidence of foliar gall psyllid (Trioza anceps). Allelopathy J 2008; 21:175-82
39. Francis F, Vandermoten S, Verheggen FJ, Lognay G, Haubruge E. Is (E)-β-farnesene the only volatile terpenoid in aphids? J Appl Entomol 2005; 129:6-11; http://dx.doi.org/10.1111/j.1439-0418.2005.00925.x
40. Riffell JA, Lei H, Hildebrand JG. Neural correlates of behavior in the moth Manduca sexta in response to complex odors. Proc Natl Acad Sci USA 2009; 106:19219-26; PMID:19907000; http://dx.doi.org/10.1073/pnas.0910592106
41. van Schie CC, Haring MA, Schuurink RC. Tomato linalool synthase is induced in trichomes by jasmonic acid. Plant Mol Biol 2007; 64:251-63; PMID:17440821; http://dx.doi.org/10.1007/s11103-007-9149-8
42. De Boer JG, Dicke M. The role of methyl salicylate in prey searching behavior of the predatory mite phytoseiulus persimilis. J Chem Ecol 2004; 30:255-71; PMID:15112723; http://dx.doi.org/10.1023/B:JOEC.0000017976.60630.8c
43. Arimura G, Ozawa R, Kugimiya S, Takabayashi J, Bohlmann J. Herbivore-induced defense response in a model legume: Two-spotted spider mites, Tetranychus urticae, induce emission of (E)-β-ocimene and transcript accumulation of (E)-β-ocimene synthase in Lotus japonicas. Plant Physiol 2004; 135:1976-83; PMID:15310830; http://dx.doi.org/10.1104/pp.104.042929
44. Van Poecke RM, Posthumus MA, Dicke M. Herbivore-induced volatile production by Arabidopsis thaliana leads to attraction of the parasitoid Cotesia rubecula: chemical, behavioral, and gene-expression analysis. J Chem Ecol 2001; 27:1911-28; PMID:11710601; http://dx.doi.org/10.1023/A:1012213116515
45. Delphia CM, Mescher MC, Felton GW, De Moraes CM. The role of insect-derived cues in eliciting indirect plant defenses in tobacco, Nicotiana tabacum. Plant Signal Behav 2006; 1:243-50; PMID:19516985; http://dx.doi.org/10.4161/psb.1.5.3279

46. Uefune M, Kugimiya S, Ozawa R, Takabayashi J. Parasitic wasp females are attracted to blends of host-induced plant volatiles: Do qualitative and quantitative differences in the blend matter? F1000Research 2013, 2:57.
47. Rasmann S, Turlings TCJ. Simultaneous feeding by aboveground and belowground herbivores attenuates plant-mediated attraction of their respective natural enemies. Ecol Lett 2007; 10:926-36; PMID:17845293; http://dx.doi.org/10.1111/j.1461-0248.2007.01084.x
48. Du Y, Poppy GM, Powell W, Pickett JA, Wadhams LJ, Woodcock CM. Identification of semiochemicals released during aphid feeding that attract parasitoid Aphidius ervi. J Chem Ecol 1998; 24:1355-68; http://dx.doi.org/10.1023/A:1021278816970
49. Quiroz A, Pettersson J, Pickett JA, Wadhams LJ, Niemeyer HM. Semiochemicals mediating spacing behavior of bird cherry-oat aphid, *Rhopalosiphum padi* feeding on cereals. J Chem Ecol 1997; 23:2599-607; http://dx.doi.org/10.1023/B:JOEC.0000006669.34845.0d
50. Erbilgin N, Powell JS, Raffa KF. Effect of varying monoterpene concentrations on the response of *Ips pini* (Coleoptera: Scolytidae) to its aggregation pherome: Implications for pest management and ecology of bark beetles. Agric For Entomol 2003; 5:269-74; http://dx.doi.org/10.1046/j.1461-9563.2003.00186.x
51. Bleeker PM, Diergaarde PJ, Ament K, Guerra J, Weidner M, Schütz S, et al. The role of specific tomato volatiles in tomato-whitefly interaction. Plant Physiol 2009; 151:925-35; PMID:19692533; http://dx.doi.org/10.1104/pp.109.142661
52. Roh HS, Park CG, Lim EG, Kim J. Acaricidal and oviposition deterring effects of santalol identiŷed in sandalwood oil against two-spotted spider mite, Tetranychus urticae Koch (Acari: Tetranychidae). J Pest Sci 2011; 84:495-501; http://dx.doi.org/10.1007/s10340-011-0377-y
53. Mayer CJ, Vilcinskas A, Gross J. Pathogen-induced release of plant allomone manipulates vector insect behavior. J Chem Ecol 2008; 34:1518-22; PMID:19031034; http://dx.doi.org/10.1007/s10886-008-9564-6
54. Gao Y, Jin YJ, Li HD, Chen HJ. Volatile organic compounds and their roles in bacteriostasis in five conifer species. J Integr Plant Biol 2005; 47:499-507; http://dx.doi.org/10.1111/j.1744-7909.2005.00081.x
55. He PQ, Zhang PY, Chen KS, Li GY. Inhibitory effects of several volatiles of Lycopersicon esculentum on Botrytis cinerea. Acta Botanica Yunnanica 2005; 27:315-20
56. van Vuuren SF, Viljoen AM. Antimicrobial activity of limonene enantiomers and 1, 8-cineole alone and in combination. Flavour Fragrance J 2007; 22:540-4; http://dx.doi.org/10.1002/ffj.1843
57. Gershenzon J, McConkey ME, Croteau RB. Regulation of monoterpene accumulation in leaves of peppermint. Plant Physiol 2000; 122:205-14; PMID:10631264; http://dx.doi.org/10.1104/pp.122.1.205
58. Pare PW, Tumlinson JH. De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. Plant Physiol 1997; 114:1161-7; PMID:12223763
59. McLeod G, Gries R, von Reuss SH, Rahe JE, McIntosh R, König WA, et al. The pathogen causing Dutch elm disease makes host trees attract insect vectors. Proc Biol Sci 2005; 272:2499-503; PMID:16271975; http://dx.doi.org/10.1098/rspb.2005.3202
60. Doares SH, Narvaez-Vasquez J, Conconi A, Ryan CA. Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. Plant Physiol 1995; 108:1741-6; PMID:12228577
61. van Wees SCM, Luijendijk M, Smoorenburg I, van Loon LC, Pieterse CMJ. Rhizobacteria-mediated induced systemic resistance (ISR) in Arabidopsis is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene Atvsp upon challenge. Plant Mol Biol 1999; 41:537-49; PMID:10608663; http://dx.doi.org/10.1023/A:1006319216982
62. Croft KPC, Juttner F, Slusarenko AJ. Volatile products of the lipoxygenase pathway evolved from Phaseolus vulgaris (L.) leaves inoculated with Pseudomonas syringae pv. phaseolicola. Plant Physiol 1993; 101:13-24; PMID:12231661
63. Dorman HJD, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 2000; 88:308-16; PMID:10736000; http://dx.doi.org/10.1046/j.1365-2672.2000.00969.x